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Twelve Month Progress Report

to

The International Isocyanate Institute

by

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Studies of Toluene Diisocyanate Induced Pulmonary Disease

Report Period: January 1, 1981 - December 31, 1981

INTRODUCTION

Our studies during the past year have been directed towards identifying isocyanate-reactive individuals for further study; challenge studies and pharmacologic follow-up of proven isocyanate reactors; and determination of the specificity of the radioallergosorbent test (RAST) with isocyanate.

MATERIALS AND METHODS

Provocative Inhalation Challenge (PIC) of TDI "Sensitive" Workers

For PIC, workers were transported to New Orleans and admitted to the Tulane Medical Center Hospital for the duration of testing. Provocative inhalation challenge was performed in the Environmental Science Research Building. The workers were exposed for 15 minutes in the environmental chamber to increasing levels of isocyanate on subsequent days, starting with a saline concentration on day one, 0.005 ppm TDI on day two, 0.01 ppm on day three, an 0.02 ppm on day four. Testing was usually halted when a 20% decrease in FEV_1 was observed or when the patient failed to react to the 0.02 ppm for 15 minutes testing, although in some cases, testing was extended to 1 hour. Prior to exposure, pulmonary function testing was performed to obtain base-line levels. Measurement of lung function was determined following the exposure; initially at 5 minute intervals for the first 15 minutes following exposure, then at 15 minute intervals for the next 2 hours, and thereafter at one hourly intervals for the remainder of the day. Prior to inhalation challenge, a blood sample was collected for cyclic AMP dose response slopes, and serum samples were collected for testing by the radioallergosorbent test (RAST) using p-tolyl (mono)-isocyanate (TMI) conjugated to human serum albumin. Where applicable,

reactors were also challenged with HDI or MDI.

Methacholine challenge was performed on a separate day to determine whether there was bronchial hyperreactivity. Subjects were tested for baseline pulmonary function to ensure that all flow parameters were within 80% of predicted normal values. Five breaths of physiologic saline were administered using a Bird Mark 3 nebulizer and lung function was again evaluated. This value was used as the base-line, prior to determining effect of methacholine upon lung function. On breath of 5 mg per ml concentration of methacholine was administered, and lung function was measured at 1.5 and 3 minute time intervals after inhalation. If a 20% drop was not observed, 5 breaths of the same concentration were administered. The procedure was continued using 10 mg/ml and 25 mg/ml concentrations. The test was stopped when either a 20% drop in FEV_1 occurred or, in the absence of an airway response, when 5 breaths of the 25 mg/ml concentration has been administered. Cumulative breath units (equivalent to one breath of 1 mg/ml) were calculated and dose response regression slopes using cumulative breath units and percent decrease in FEV_1 were graphed for each subject.

Lymphocyte Cyclic AMP Dose Response Slope Studies

For this assay, lymphocytes were extracted from 50 ml of peripheral blood by Ficoll-Hypaque density gradiant centrifugation. Cells were checked for viability and one million lymphocytes were incubated with varying concentrations of isoproterenol in Hank's balanced salt solution (HBSS), and the isocyanate in dimethylsulfoxide (DMSO). Cells were washed, precipitated with trichloroacetic acid, freeze-thawed to disrupt cells, and extracted with water saturated ether. Cyclic AMP levels were quantitated using a standard radioimmunoassay method. The number of picoMoles

of cAMP were obtained from a standard graph and, for evaluation, were expressed as percent stimulation of cAMP above base-line. Dose response regression lines were determined using the linear ascending portion of this slope.

Enumeration of β-adrenergic Receptor Sites

The principle of the test to quantitate the number of β-adrenergic receptors is to use ^{3}H alprenolol, a potent β-antagonist, and a non-labelled antagonist, propranolol, to complete for available binding sites on lymphocyte surfaces.

Briefly, lymphocytes were separated from 50 ml peripheral venous blood by the density gradient centrifugation method. Lymphocytes were counted and adjusted to 4×10^{6} cells per 100 µl buffer for each sample tube. The tubes were assayed in quadruplicate at the same time. Cells were incubated with ^{3}H alprenolol alone to give the total binding value. In addition, cells were incubated with appropriate concentrations of propranolol in sufficiently high concentrations to block the binding of the alprenolol, and permit determination of non-specific binding of the labelled material. All lymphocytes were incubated for 5 minutes at 37°C and the reaction was stopped by the addition of cold saline. The contents of the tubes were collected on paper discs by filtration using a sampling manifold. Filter paper discs were washed twice to elute any unbound ^{3}H alprenolol then transferred to scintillation vials and allowed to dry overnight. The following morning, liquid scintillation counting fluid was added to the vials and they were counted on a Beckman Liquid Scintillation Counter.

It has been determined that 75 ± 12 fmol of alprenolol bound to 2×10^7 cells corresponds to 2,000 β-receptors per cell. To determine the femtomoles (fmol) of alprenolol bound to the lymphocytes, the non-specific binding value was subtracted from the total binding, and the number of receptors calculated.

RADIOALLERGOSORBENT TEST (RAST)

Preparation of Hapten Conjugates:

Isocyanates used to prepare hapten conjugates were p-tolyl monoisocyanate (TMI), xylene diisocyanate (XDI), hexamethyl diisocyanate (HDI), and diphenyl methylene diisocyanate (MDI). MDI was recrystallized prior to use.

For the conjugation, 280 µl of TMI, XDI or HDI was added dropwise to 100 ml of a 1% solution of human serum albumin (HSA) in borate buffer, pH 9.4 (0.05 M boric acid, 0.05 M Cl, 0.035 M NaOH), at 4°C. The mixture was maintained at 4°C for 30 minutes with stirring, then centrifuged at 1500 rpm for 15 minutes. The mixture was left undisturbed for 1 hour at 4°C, then centrifuged. The supernatant was dialized for 12 hours each against two changes of physiological saline followed by four changes of distilled water. The retentate was lyophilized and stored at -20°C.

For MDI, 265 mg was dissolved in 10 ml p-dioxane by sonication for 30 minutes at room temperature. This was added dropwide to 50 ml of 1% HSA, then treated as above.

Conjugation was confirmed by UV spectroscopy and electrophoresis.

RAST:

For RAST, hapten conjugates were coupled to CNBr activated filter paper discs by addition of 10 mg/ml hapten-HSA in borate buffer, pH 8.0,

for 6 hours on a rotator at room temperature. Following incubation, discs were washed three times in assay buffer (500 ml 0.2 M tris buffer, pH 7.5; 500 ml 1.8% NaCl (w/v); 10 ml 5% NaN_3; 5 ml Tween 20; 2 gm BSA) and stored in assay buffer at 4°C until use.

For the test, 100 µl serum was added to tubes containing a hapten-conjugate coupled disc or a HSA-coupled control disc, incubated overnight at room temperature on a rotator, and washed three times each with 2.5 ml saline to remove unreacted serum. One hundred ml ^{125}I-labelled anti-IgE (approximately 40,000 cpm) was added, tubes incubated overnight at room temperature on the rotator, then washed three times in saline, and counted for 5 minutes on a Beckman Biogamma counter.

RAST ratio was determined by dividing cpm obtained with the test disc by cpm obtained with the HSA control disc. All tests were run in duplicate.

Inhibition Studies

To determine cross reactivity, 100 µl of PBS or 10 mg/ml solution of each hapten conjugate in phosphate buffered saline (PBS) was incubated with 100 µl serum for 2 hours at room temperature. The mixtures were centrifuged at 2000 rpm for 15 minutes and 100 ml of supernatant used for RAST as described above.

Carriers

Other carriers used for the conjugation were: purified human serum albumin (HSA: Miles Pentex Laboratory, Kankakee, IL), ovalbumin, Grade VI (OA: Sigma Chemical Co., St. Louis, MO), ribonuclease A from bovine pancreas, Type I-A (RIB.A: Sigma Chemical Co., St. Louis, MO), poly-l-lysine hydrobromide, Type V (LYS: Sigma Chemical Co., St. Louis, MO) and serum from a normal subject with no history of isocyanate exposure (NHS). The

protein content of the NHS was determined by the Lowry method; the concentration was calculated from a standard curve prepared using human serum albumin (Miles Pentex Laboratory, Kankakee, IL). The amount of NHS used in coupling procedures corresponded to a 1% solution of human serum albumin in 100 ml of isocyanate coupling buffer.

Preparation of Isocyanate-Protein Conjugates

All isocyanate-protein conjugates were prepared by a standard protocol. Antigens were prepared by coupling the four isocyanates to HSA, OA, RIB.A, LYS or NHS. Briefly, the method consisted of dropwise addition of isocyanate to a stirred 1% solution of protein carrier in buffer at pH 9.4. The mixture was stirred on ice for 30 minutes, centrifuged in glass centrifuge tubes for 15 minutes at 2,250 rpm, then left undisturbed for 1 hour at 4°C. Conjugates were isolated by lyophilization after extensive dialysis at 4°C. Conjugation was confirmed by ultraviolet spectroscopy and electrophoresis.

Spectrophotometric Analysis

The optical density of proteins and TMI-protein conjugates was recorded using a Beckman Model 25 spectrophotometer. Conjugates were dissolved in phosphate buffered saline (PBS). To determine the moles of TMI linked to the corresponding carrier proteins, optical density analysis of the conjugates was undertaken. A conjugate of p-tolyl isocyanate-protein and p-tolyl isocyanate-ε aminocaproic acid was prepared. Absorption standard curves of solutions of TMI-protein, protein, and TMI-ε amino-caproic acid were prepared using four different concentrations of the constituents, to determine the molecular ratio of TMI to protein. Molar absorptivity of the substance was calculated using the formula: $E = A/bc$, where: A = observed absorbance; b = 1 cm light path; C = concentration of the substance in moles per liter. Solving these equations:

$$A_{w_1} = E_{xw_1} b C_x + E_{yw_1} b C_y$$

and

$$A_{w_2} = E_{xw_2} b C_x + E_{yw_2} b C_y$$

where: i.e. : A_{w_1} = absorbance at 245 nm due to TMI-HSA,

A_{w_2} = absorbance at 280 nm due to TMI-HSA,

E_{xw_1} = molar extinction coefficient of HSA at 245 nm,

E_{yw_1} = molar extinction coefficient of TMI-ε-aminocaproic acid at 245 nm,

E_{xw_2} = molar extinction coefficient of HSA at 280 nm,

E_{yw_2} = molar extinction coefficient of TMI-ε-aminocaproic acid at 280 nm,

w_1, w2 - wave lengths,

C_x = concentration of HSA in moles per liter,

C_y = concentration of TMI in moles per liter,

the equations become:

$1.640 = 40{,}300\ C_x + 13{,}250\ C_y$ and $0.192 = 30{,}000\ C_x + 720\ C_4$

From these equations the molecular ratio of TMI to HSA was calculated.

Preparation of Acetic Anhydride-Protein Conjugates

Acetylation was performed by treating 3% protein solution in half saturated sodium acetate with 1.8 ml acetic anhydride for 4 days at 4°C. The conjugate was dialysed against distilled water to remove buffer and unreacted material.

Electrophoresis

Electrophoresis was used to evaluate the conjugate and the carrier mobilities. Microscope slides (70 x 50 mm) were coated with 5.5 ml of 1% agarose (Eastman Organic Chemical, Rochester, NY) in barbituric acid buffer, pH 8.6. Ten micro-liters conjugate or control protein carrier (10 mg/ml PBS) was placed into wells (4 mm in diameter) close to the cathode. Wicks (100 x 100 mm) wetted with buffer were placed on polar ends of the gel, followed by electrophoresis for 45 minutes at 10 volts/cm current. The slides were removed, washed with saline, dried and stained with Coomassie Brilliant Blue for 10 minutes, then clarified with destain solution (450 ml 96% ethanol, 100 ml glacial acetic acid, 450 ml deionized water).

RESULTS

Provocative Inhalation Challenge Studies:

The first individual (AG) had worked for seven years in a plant manufacturing TDI. Prior to this period he had no previous respiratory problems although it is possible he suffered from mild rhinitis for a few years before he began working at the plant. One month after he began working at the plant he noticed his first attacks of tightness of the chest and wheezing. There was no accompanying rhinitis. At that time he was working in the maintenance department and was exposed to both TDI and TDA. This

continued for some 4 years. Following any spills of TDI he would also develop coughing within a few minutes and, if the TDI level was high, he also developed wheezing and shortness of breath. He reported approximately half a dozen acute attacks, in all, which prompted him to seek first aid in the medical department. These acute attacks generally cleared up in one or two hours and never recurred once he ceased exposure and returned home. However, on occasion, he reported spontaneous development of shortness of breath and wheezing after returning home when there had not been any obvious immediate attacks earlier in the day. In addition to these acute attacks which were sufficient to cause him to seek medical attention, he estimated there had been 20 to 80 minor attacks per year. He said he could not remember details, but every one in the plant had the same problem. When he was moved from the isocyanate areas his symptoms regressed; however, when he was sent back to do jobs involving TDI exposure, from time to time, his symptoms recurred. For the last six months before our testing he had noticed mild attacks, particularly of coughing, 2 or 3 times a week even without obvious isocyanate exposure. Finally at the time of a TDI plant turnaround three weeks before we saw him, he developed a particularly bad attack.

He was challenged on the first day we saw him with saline control, on the second day with TDI 0.0185 ppm for 15 minutes, and on the third day with TDI 0.019 ppm for 60 minutes. No obvious positive responses were obtained although he did complain of minimal symptoms for 3 to 4 hours post challenge with 1 hour of isocyanate. Challenge with methocholine showed that he had mild, non-specific broncho-hyperresponsiveness, the number of units required to induce a response being 168 prior to the TDI challenges and 118 units when the TDI tests were completed. This mild change is of doubtful signficance; however, non-specific hyperresponsiveness is known to increase following

late asthmatic reactions. Cyclic AMP dose response studies on this individual were normal. RAST ratios were not elevated.

The second individual (S.P.) worked for a petroleum refinery for 16 years, primarily as an asphalt stillman. In November of 1979, he was seconded to a newer operation producing wax/carbon copying paper. His work involved mixing TDI with the wax and he was required to tip 5 gallon buckets of TDI into an electric pump for the first 6 weeks of the operation. He and a colleague would daily pour a total of approximately 300 gallons of TDI into the pump over a period of time. There apparently were spills and subsequent continuous exposure to the wax-TDI-amine mixture used in the process. In retrospect, he believed he noticed some shortness of breath and wheezing from the very first day he was involved in this process. One to two weeks after the process was begun, an amine was inadvertently mixed with the TDI and the chemical mixture erupted, giving off a dense cloud which contaminated the whole building. At this time, he and most of his colleagues were affected by shortness of breath and a "cold." From then on, he had symptoms which occurred within minutes of TDI being poured and persisted throughout the work period, which frequently necessitated him returning home during the shift. Once removed from the work environment, however, his symptoms regressed quickly and he remained well at home. Once he suspected he had become affected by the isocyanate, he began to work outside of the processing building and remained well whenever he was upstream from TDI. However, when the wind direction changed, he felt the symptoms occurred within 5 minutes and, on one occasion when a colleague came into his hut with TDI contaminated clothes, he had a relapse within 5 minutes. In March 1980, the process was closed down and 2 weeks later he felt fully recovered. In May 1980 however, a truck carrying TDI was unloaded after

which the residue in a hose discharged onto the ground around his hut. He estimated the total spill involved approximately 5 gallons. This made him immediately ill and he returned home. Although he did not deteriorate to such an extent that he required hospital assistance, he has not returned to the plant. His asthma, however, has continued and was particularly bad for the 2 weeks following his last day of work. During this period he awoke many times at night, requiring the use of a bronchodilator inhaler. Asthma was formally diagnosed by a chest physician in June and has been treated with Theophyllin and Prednisolone and a bronchodilator ever since. When the Prednisolone was discontinued three to four months after his treatment began, his symptoms recurred. When admitted to Tulane for testing, he was taking 20 mg of prednisone on alternate days, Slophylline 1 g daily, a bronchometer aerosol and a Vanceril inhaler 4 to 8 times daily. While in New Orleans all medication was withdrawn without relapse during the first week of his admission and inhalation provocation tests were commenced. Methacholine inhalation tests showed moderate broncho-hyperresponsivity. FEV_1 fell more than 20% after 10 methacholine inhalation units. He was challenged on subsequent days with water and with a TDI concentration of 0.0045 ppm for 15 minutes. The results of these tests showed a typical late asthmatic reaction to the TDI exposure. Leukocyte counts carried out in association with the inhalation challenge tests with TDI showed that the eosinophil count rose by 57%. Methacholine challenge on the day following the TDI reaction showed the response to now occur with 5 methacholine inhalation units. RAST with TMI-HSA and MDI-HSA were both negative. The cyclic AMP sample was lost in a lab accident.

The third individual (G.F.) began working for a telephone company

July 1979. He generally worked outside and was frequently involved in preparing polyurethane insulation material for underground cables. This involved mixing polyol with MDI which he did 2 to 10 times each month. Apart from occasional headaches, which he attributed to exposure to the vapors, he had never been aware of any respiratory or allergic problems until an episode in April of 1980. On this day, he was on the surface mixing the two encapsulant components while his workmates were in the hole. He prepared about 12 gallons in the space of 15 minutes. He recalls no accidents and no spills and he does not believe there was contamination of his clothes or his skin by direct contact. He lowered the prepared mixture to his workmates and during the next 10 minutes he noticed some itching on his face followed quickly by similar itching on his limbs and trunk. He initially thought he was developing sunburn but when his workmates emerged some 20 minutes after the symptoms began, they noticed that his face was swelling and his eyes were closing. He was driven to the hospital, the journey taking approximately 30 minutes, during which time he felt faint and, on arrival, was only partially conscious, requiring assistance from nurses at the hospital in order to walk from the car to the emergency room. By this time he looked like a "Michelin" man and was unable to see because his eyes were completely closed. The nursing staff were unable to remove his boots because his legs and feet were swollen. He received injections and made a steady and full recovery in the ensuing 60 minutes.

He underwent 3 inhalation challenge tests with MDI in addition to control tests with saline. One of the challenge tests involved mixing the regular reactants for the polyurethane encapsulant in a confined exposure

chamber. He did this for 15 minutes and atmospheric monitoring showed a mean concentration of MDI of 0.0007 ppn. On subsequent days, he was exposed to higher concentrations of atmospheric MDI generated by heating MDI in a stream of dry nitrogen. The concentrations generated were measured by HPLC at 0.0016 ppm and 0.0091 ppm respectively. No respiratory response was seen following these exposures.

To exclude the possibility that direct skin contact might have induced the reaction, he spent ten minutes washing his hands in the polyl component of the encapsulant mixture. After a further five minutes, he washed his hands thoroughly. No local or generalized response was noted over the ensuing 30 minutes and so a further "washing" challenge was performed using the same technique with MDI. Again no generalized or local response was seen and no change in ventilatory function occurred. Routine skin prick test with insect venom was negative. The fire ant prick test concentration of 1 in 10,000 proved to be negative but an intradermal test at this strength was strongly positive. The intradermal test at 1 in 100,000 was negative. None of the bee, wasp, or hornet venoms showed a reaction, even at concentrations of up to 1 in 1,000. Methacholine challenge was negative. RAST was negative. cAMP was normal. This individual was felt to be a non-reactor who had most likely experienced an anaphylactic response to fire ant bite.

A fourth individual (R.M.) was tested at Tulane Medical Center in May, 1981. He had worked for some years for one of the large chemical companies. It was his job to visit other companies which used products produced by his firm to make polyurethane foams. He advised on modifications regarding the manufacturing process whenever difficulties arose with the quality of the end product. He estimated that he visited between 4-6 of these manufacturing plants each month. Of the 60 annual visits that he made, probably only 5 or 6 actually involved his exposure to the manufacturing area itself

and to isocyanates. He reported that in 1974 he developed a cough which was at times productive of small quantities of mucopurulent sputum. During 1975 to 1978 the attacks became more frequent and more severe, occurring from 5 to 10 times each year, between which he was generally well. He could not recall whether he had wheezing or shortness of breath at the time, but he did relate that he was treated with theophyllin through most of this period and corticosteroids for six months. He could not recall whether these medications were effective or not. During 1979 he became unduly short of breath associated with wheezing. This initially was intermittent, but within a few months the symptoms became persistent although their severity varied. He learned to relate worsening symptoms with occupational exposure to TDI. During 1979 he became certain that it was TDI exposure which produced immediate shortness of breath, wheezing and irritation of the eyes. There were no nasal symptoms. He had 3 particularly bad attacks during November and December of 1979 when he visited 3 different plants in close succession and had not fully recovered from the symptoms generated by the first visit before the subsequent visit began. After the third attack, he was feeling moderately unwell but was not admitted to hospital. He did, however, avoid further factory visits involving TDI exposure until June, 1980. During this period he had no further acute exacerbations but continued to feel short of breath with wheeze.

He underwent methacholine inhalation challenge tests giving a 15% decline in FEV_1 after the maximum cumulative dose of methacholine (640 units), indicating minimal non-specific bronchohyperreactivity. He subsequently underwent provocative inhalation challenge test with steadily increasing doses of TDI, receiving 15 minutes of 0.005 parts per million on the first day; on subsequent days, 0.01 and 0.02 ppm for 15 minutes, with a final exposure of 0.02 ppm for 60 minutes. He noted no symptoms during the control day

but did remark feeling chest tightness during the afternoon and evening of each TDI challenge day. There was some evidence of a late reaction commencing at 6 hours on the day of the last TDI challenge for 60 minute Further methacholine challenge, following completion of the TDI provocative challenge, showed that he now reacted to the cumulative dose of 320 units. The findings suggested that this individual probably had a delayed onset asthma brought on by TDI. RAST test with TMI-HSA gave a ratio of 1.49. cAMP dose responses were normal.

A fifth individual (M.S.) had worked most of his life as a spray painter, principally involved in spraying vehicles. He had no trouble medically until 1974 when he began working for a large company. In this particular job he worked in the new paint shop where he was the only spray painter. The shop was poorly equipped but spraying was carried out with the aid of a protective respirator with outside air supply. The job involved preparatory body work prior to spray painting for which he used epoxy resins which he both applied and sanded. At times he would spray for eight hours daily, but on average he probably spent a quarter to a third of his time actually spraying. During 1974 he became aware of attacks of shortness of breath accompanied by chest tightness and wheeze. There were no nasal problems. He related these attacks to his occupation of spray painting but not to the preparatory work. These attacks would come on immediately after he began spraying and would generally get worse at night when he was in bed, frequently disturbing his sleep. Similar attacks could persist for a week before full recovery occurred, even without further exposure. The dry cough sometimes persisted even longer. He attributed the symptoms to three particular paints, each of which had an activator of hardener which, on approaching the companies, was learned to be a dimeric hexamethylene diisocyanate. During the years

from 1975 to 1979 his symptoms gradually worsened. Though he tried different paints and various different respirators, he achieved no real relief until he stopped using the activators and hardeners. He found that, without this, the paint dried less quickly and had a less hard finish but was nonetheless reasonably satisfactory. Once he adopted this procedure his symptoms improved and attacks became less frequent; however, he became aware of a few attacks which he could not obviously relate to work. Some were related to exercise but had no previous provoking factors.

The paint shop was closed down in March 1981, and since that time he has been out of work and has been aware of only 2 episodes of wheezing and breathlessness. We challenged him with HDI beginning with 0.0034 ppm for 15 minutes and increasing to 0.0167 ppm for 15 minutes. Finally we challenged him with 0.007 ppm for 60 minutes. We were unable to induce a bronchoconstriction response in him although his history was, overall, a convincing one; however, dimeric HDI was used specifically in his occupations, and we are unable to use this in our present provocative challenge testing situation. It is felt by our inductrial hygienist that the dimer from spraying operations could predominate as an aerosol and that quite larger quantities could be present. Challenge with methacholine induced a 20% decline in FEV_1 after 340 units. Subsequent challenge with methacholine following all of the HDI exposures gave a 20% decline in FEV_1 with 360 units. Thus, the individual had a mild bronchohyperresponsiveness which did not change signficantly following HDI exposure. RAST with TMI-HSA and HDI-HSA were normal. cAMP dose responses were normal.

We are continuing to do follow-up studies on JM, who had worked as a pipe fitter at a company manufacturing TDI since 1973. Toward the end of his first year with that company he noticed two to four minor attacks of

chest tightness with wheezing which did not cause distress and he sought no medical aid. Subsequently, he began working in a rotating position with a maintenance shift which worked intermittently in the TDI area. He had respiratory attacks which lasted no more than one-half hour. After one year he began working on a regular day shift in TDI manufacture and soon after developed moderately severe type wheezing some 2 hours after beginning work. There was also cough, tightness of the chest and an undue shortness of breath. He sought medical aid and was treated with oxygen bronchodilators. He was not aware of obvious TDI exposure. Since that time he has had dozens of episodes of tightness of the chest within 15 to 30 minutes of TDI exposure, which has necessitated frequent visits to the sick bay. His cough often persisted longer, sometimes as much as 24 hours, and would be productive with mucoid sputum which at times was yellow in color. Since recognizing a close relationship between TDI exposure and the attacks, he claims he has never experienced an attack without being aware of TDI odor. Some of these attacks, however, have involved apparently trivial degrees of exposure, since some have occurred several hundred yards away from the TDI zone, which he was downstream from prevailing winds. Because of his sensitivity he was finally moved to the most remote location away from the TDI manufacturing area.

At the time that we first tested him in March, 1980, he had been away from TDI exposure for three weeks and was completely free of symptoms. Methacholine challenge testing at this time showed a significant fall in FEV_1 following three inhalations of 5 mg/ml methacholine. This was followed on the following day with challenge with 0.005 ppm TDI for 15 minutes.

A quite marked immediate asthmatic reaction was provoked, with FEV_1 falling from 4.45 liters to a minimum level of 2.2 (a decrease of 51%), 25 minutes after exposure commenced. Recovery from this reaction was complete within 2 hours, but after 3 hours, there was some evidence of a mild late reaction. In order to evaluate whether there might be cross reactivity between isocyanates, we also challenged this individual with 15 minutes of HDI, at a concentration of 0.005 ppm. No symptoms were induced and there was no evidence of an asthmatic reaction.

This individual was followed up on 3 further occasions: February 1981, July 1981, and December 1981. When seen in February he reported he had not had any more asthma attacks after working away from the TDI area. The methacholine challenge test results showed that non-specific bronchial hyperreactivity had not changed significantly. He still reacted strongly when challenged with 0.006 ppm TDI for 15 minutes.

When rechallenged in July, it now took a TDI concentration of 0.011 ppm for 15 minutes to induce an equivalent bronchospastic response. In November of 1981, he was still giving a definite immediate asthmatic response to 15 minutes exposure to TDI around 0.01 ppm. On this occasion we also tested him not only with the usual mixture of 2,4 and 2,6 isomers, but also with the pure 2,6 isomer. He responded to both in an identical fashion. There were, however, on these occasions no evidence of a late reaction, suggesting further lessening of reactivity. Repeat of the methacholine challenge test, both before and after investigations, showed identical levels of nonspecific bronchial hyperreactivity. This sensitivity does not appear to have altered significantly since we first saw him.

RAST with TMI-HSA on the serum sample collected in February, 1981 was not performed. The ratio obtained on the June, 1981 sample was 2.22. The November, 1981, serum RAST ratio was 1.52.

cAMP dose responses with TDI, and isoproterenol were within normal limits for the February, June and November samples.

When first seen in March, 1980, the β-adrenergic receptor numbers were reduced by approximately 25%. The receptor numbers were all within normal limits on the February, June and November 1981 samples.

Spectroscopic analysis

The molecular ratio of TMI to HSA was approximately 32.1. It was not possible to calculate the number of moles of reagent bound to protein for the other conjugates. However, for all the conjugates used in this study, analysis in the ultraviolet region showed differences between the absorption curve of the proteins and the absorption curve of isocyanate-protein complexes. These differences reflect changes in the protein molecule and were consistent with a reaction between the isocyanate and the protein. Spectroscopic examination of hexamethylene diisocyanate-protein conjugates was the only case which gave no change in the absorption curve curves. This agreed with the fact that few aliphatic compounds give an absorption band in the region between 220 and 290 nm. The assumption that a chemical alteration in the protein molecule occurred as a result of the action of different isocyanates was, however, supported by electrophoretic assay on our conjugates.

Electrophoretic studies

The results of the electrophoretic studies are shown in Figure 1. All conjugates showed increased anodic migration with the exception of Ribonuclease A, which showed cathodic mobility.

EVALUATION OF THE RADIOALLERGOSORBENT TEST (RAST)

During this year we also completed the evaluation of RAST results on serum samples collected during the final epidemiological survey visit to the Olin plant in Lake Charles. Our results showed that 7 of 149 workers gave a positive RAST test. One of these 7 individuals had a probable history of TDI sensitivity and one had a rash which was possibly caused by TDI exposure. All the remaining 5 were asymptomatic and no evidence of isocyanate induced disease could be found. A manuscript was prepared and sent to III prior to being submitted to the journal "Clinical Allergy" for publication.

We also evaluated RAST positive sera to determine whether there was cross-reactivity between different isocyanates (TMI, HDI, MDI, XDI) conjugated to human serum albumin. The results of inhibition studies using the different isocyanate-HSA conjugates showed that the antibodies present in the serum from the TDI workers reacted with conjugates of human serum albumin with isocyanate other than TMI Table I. They did not, however, give a clear cut RAST inhibition pattern, as would be expected if there is cross-reactivity. The suggestion was raised that these antibodies may be showing some degree of specificity for the carrier molecule. We therefore prepared conjugates of TMI, XDI, MDI and HDI with ovalbumin, ribonuclease, lysine and normal human serum. The results of these studies are shown in Table II. The lack of positive results with the ovalbumin and the ribonuclease-isocyanate conjugates were strongly suggestive of carrier specificity of the antibody rather than hapten specificity (i.e. the antibodies are directed against altered protein rather than against the isocyanate portion of the complex).

To test this, we attempted to alter human serum albumin using acetic anhydride. Acetic anhydride reacts with the NH_2 group of lysine in the

protein in a similar way to that which is thought to occur with isocyanates. When the RAST test was carried out using a TMI-HSA positive antisera with this acetic anhydride altered human serum albumin, a strong positive was obtained with one of the worker's serum sample. The results of inhibition studies are shown in Table III. These studies confirm the possibility of carrier specificity of the antibodies. This, together with the other findings, is strong evidence that the antibodies in the RAST are indeed carrier, rather than hapten, specific. A manuscript is in preparation describing these findings.

Cold air studies

The studies comparing cold air challenge with methacholine challenge showed that there was a reasonable correlation when the cold air was delivered in conjunction with hyperventilation. There are, however, major problems with the cold air method in that it is not possible to control the severity of the bronchoreaction as can be done with methacholine. We have therefore stopped doing these tests and do not feel that it would be an appropriate test to do at the worksite.

Table I. Example of Cross Reactivity Testing Using RAST Inhibition

Pre incubation	Post incubation RAST ratio with TMI-HSA	HDI-HSA	MDI-HSA	XDI-HSA
TMI-HSA	0.78	0.60	0.62	0.86
HDI-HSA	0.75	0.16	0.51	0.19
MDI-HSA	0.35	0.25	0.09	0.26
XDI-HSA	0.63	0.15	0.52	0.07

Numbers indicate $\frac{\text{RAST after pre incubation of serum}}{\text{RAST before pre incubation of serum}}$

Thus, numbers approaching 1 = no inhibition and numbers approaching 0 = inhibition

Table II. Results of the radioallergosorbent testing in the sera of isocyanate reactors and in the sera from controls.

RAST INDEX

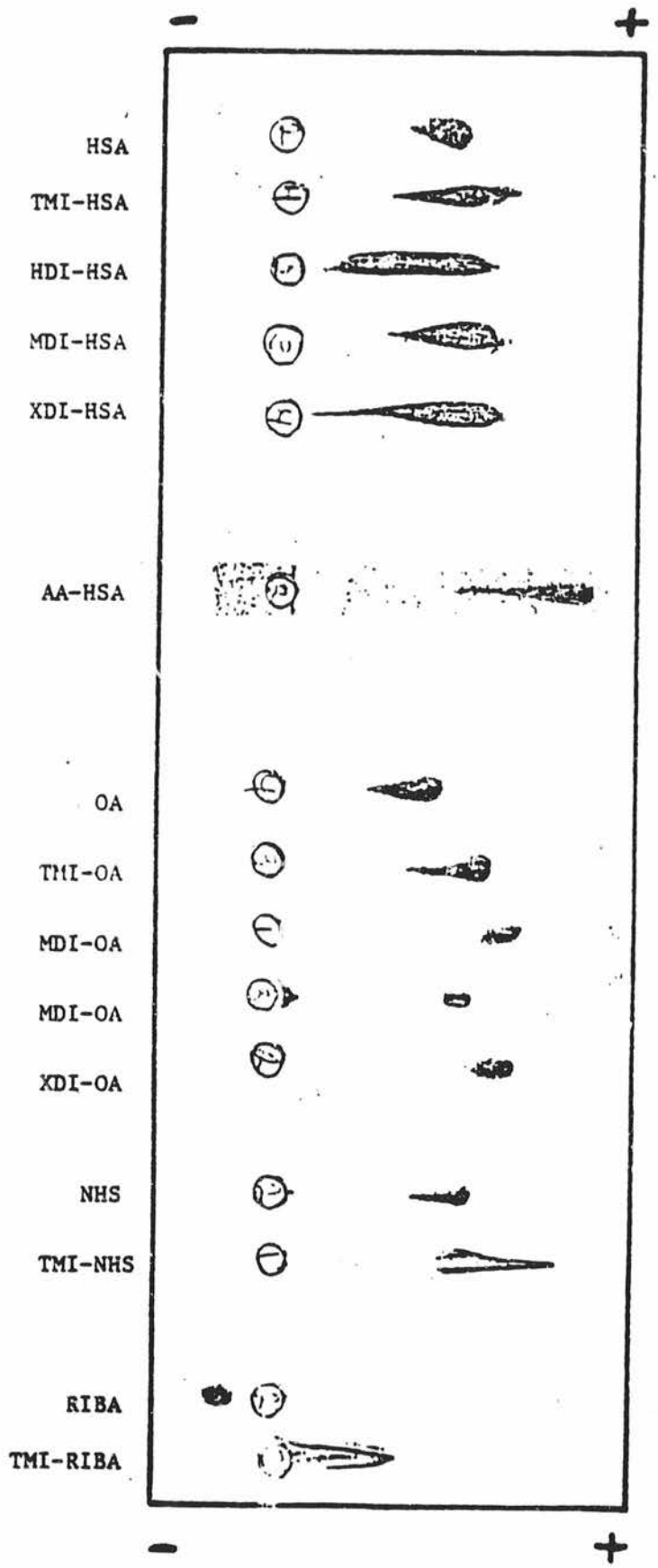
Worker	TMI-HSA	MDI-HSA	XDI-HSA	HDI-HSA	TMI-OA	MDI-OA	XDI-OA	HDI-OA	TMI-NHS	MDI-NHS	XDI-NHS	HDI-NHS	TMI-RIB.A	XDI-RIB.A	HDI-RIB.A	TMI-LYS.	AA-HSA
1	1.71	3.05	3.95	4.10	1.71	1.67	2.82	1.56	2.96	2.58	2.22	4.21	3.44	2.22	3.29	1.18	1.78
2	4.94	2.42	7.22	6.89	1.50	1.13	1.28	1.44	3.12	1.92	4.64	4.26	3.01	1.44	2.18	1.06	1.54
3	42.26	25.68	38.59	35.33	3.21	1.21	1.89	1.44	24.70	35.93	36.67	36.69	4.55	1.96	3.14	1.10	15.57
4	1.80	1.45	1.52	1.67	1.21	0.94	1.02	0.89	2.63	1.59	1.37	1.71	2.21	1.43	2.19	0.89	0.98
5	24.3	21.25			1.63	1.66											
Control																	
1	1.27	1.39	0.99	1.01	1.52	1.32	0.85	0.83	1.92	0.98	0.90	0.85	2.67	1.10	0.96	1.21	1.11
2	1.68	1.63	1.39	1.46	1.47	1.21	1.03	0.93	2.26	1.59	1.11	1.05	2.86	1.19	1.31	1.21	1.26
3	1.37	1.32	1.22	1.14	2.06	1.47	1.30	0.94	2.23	1.05	1.02	0.94	3.63	1.34	1.52	1.25	0.99
4	1.36	1.25	0.97	0.95	1.42	1.18	1.17	0.85	2.33	1.02	0.93	0.86	2.68	1.07	0.89	1.06	0.92
5		1.72			1.33		1.14	0.82									

Table III. RAST inhibition studies with Acetic Anhydride altered HSA (AA-HSA)

Pre-incubation of serum with	Post incubation RAST Ratio		
	TMI-HSA	MDI-HSA	AA-HSA
Saline	62.7	27.8	28.6
HSA	61.4	30.1	29.6
TMI-HSA	*	*	2.4
MDI-HSA	*	*	2.0
AA-HSA	70.6	29.6	2.4

*See Table I.

Figure 1. Electrophoresis of protein-isocyanate conjugates



Project NA-B 10

Comments on the annual report by Butcher of January 1982

1. In the case of a bronchial provocation 2,4 TDI had the same effect as 2,6 TDI.

2. The determination of the bronchial hyperreactivity in connection with cold air has in the meantime been discontinued because of dosage problems. (!)

3. In the part describing methods B. mentions a method of determining the beta-adrenergic receptor sites; however, no results were presented.

4. During the period convered by the report 5 patients with potential hypersensitivity were examined. When relating this to the research subsidy paid by III, the project appears to be rather impractical. Moreover, no final diagnosis is given for several of the case histories.

5. For case 2 an exorbitant increase of the eosinophilene was observed after PIC; any comments or interpretation of this diagnosis are missing: have such phenomena been observed frequently? May this be taken as a criterion for any immunological hypersensitivity?

6. Case 4 is taken by Butcher as a hypersensitivity of delayed type, although cAMP was negative?! (From this may be deduced that cAMP appears to have no appreciable importance.)

By Professor Dieter

3/24/82.

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March 25, 1982

Dr. Dennis C. Allport

Polyurethanes Research and
Application Research Manager
A.R.T.S. Dept., Organics Division
Imperial Chemical Industries PLC
PO Box 42, Hexagon House
Blackley, Manchester
M9 3DA, England

Dear Dr. Allport :

The following is the comments of FE Toxicology and Occupational Health Subcommittee on the final report for I.I.I. Project B-10-NA-4 titled " Studies of Toluenediisocyanate Induced Pulmonary Disease ".

1. Methacholine inhalation challenge seems not to be effective method to identify the isocyanate-sensitive personnel, and it is not an acceptable method in Japan from the Japanease public common sense.
2. The result of this study seems to be somewhat different from that of Dr. Karol's study, because some correlation was found between TDI sensitization and RAST positiveness in Dr. Karol's study, while almost any correlation was not observed between them in this report. This is a questionable point.

With warmest personal regards,

Sincerely yours,

Susumu Inoue

Susumu Inoue

Chairman, FE Toxicology and
Occupational Health Subcommittee

cc : Dr. Sumi
FE Subcommittee Members

APPENDIX C

TULANE UNIVERSITY
School of Medicine
NEW ORLEANS, LA. 70112

JUN 10 1982

Department of Medicine
Clinical Immunology Section
1700 Perdido Street
(504) 588-5578

June 4, 1982

Arthur Chivvis
Managing Director
International Isocyanate Institute
P.O. Box 1268
New Canaan, Connecticut 06840

Dear Art:

This letter is to address some of the queries raised regarding the progress report, as requested by Dr. Allport.

In answer to Professor Diller's comments: We have performed beta receptor determinations on patient JM. Initially this patient had reduced receptor numbers. On the three subsequent testings the number of receptors were all found to be within normal limits.

Regarding the final diagnosis for the individuals who underwent provocative inhalation challenge: The first patient probably does not have TDI reactivity but we cannot completely exclude this possibility. The second patient has late onset asthma induced by isocyanate. The third individual does not have isocyanate induced asthma and the reaction observed was almost certainly due to being bitten by a fire ant. The fourth individual has a probable delayed onset asthma induced by isocyanate. The fifth individual probably is not sensitive to isocyanates although we cannot completely exclude the possibility, in view of the likelihood that polymeric isocyanates may be present in high concentration in the spraying operation. The sixth individual is a confirmed TDI sensitive individual. We are following him longitudinally to determine whether he loses his TDI and methacholine reactivity.

Case number 2 was the only case where we saw a marked increase in total peripheral blood eosinophil count. An increase in blood eosinophils is often associated with asthma. We have, however, been looking at all challenge study patients for the total eosinophil numbers and the eosinophilia noted in case number 2 has not been a common finding in TDI reactive individuals.

Case number 4 is indeed probably a delayed asthma. The comment regarding cyclic AMP is well taken. Most of the individuals we have tested have been immediate reactors and, in these individuals, we have seen decreased cyclic AMP levels. A greater number of individuals with immediate,

delayed, or late onset isocyanate induced asthma need to be examined before any conclusions can be drawn regarding the importance of adrenergic effects of isocyanates.

In reply to the comments of Doctor Inoue: A possible difference between our findings and those of Doctor Karol is that we are performing provocative inhalation challenge on our study subjects to confirm sensitization to TDI. I understand Doctor Karol's study subjects are considered sensitive only on the history. As may be noted from some of the individuals that we have tested by provocative inhalation challenge, workers can present with a reasonably strong history indicative of TDI sensitivity, yet upon challenge, prove to be non-reactive. This could very much affect the findings regarding use of the RAST test as a diagnostic procedure.

I hope these comments help to clarify the points raised regarding the progress report.

Yours sincerely,

Brian T. Butcher, Ph.D.
Associate Professor of Medicine

BTB/gw

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